HYPOTHALAMUS-SPECIFIC POLYPEPTIDES

REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application Ser. No. 60/1023,220, filed Aug. 2, 1996, which is explicitly incorporated by reference, as are all references cited herein.

GOVERNMENTAL RIGHTS

[0002] This invention was made with governmental support from the United States Government, National Institutes of Health, Grants GM32355 and NS33396; the United States Government has certain rights in the invention.

FIELD OF THE INVENTION

[0003] This invention relates to the identification, isolation, sequencing, use, and expression of hypothalamus-specific proteins and fragments thereof.

BACKGROUND OF THE INVENTION

[0004] The hypothalamus, a phylogenetically ancient region of the mammalian brain, is responsible for the integration of the central nervous system and the endocrine system and is particularly related to the physiological response to stress. In contrast to laminar cortical structures such as the cerebellum and hippocampus whose final functions rely on innervation from the thalamus and brain stem, the hypothalamus is organized as a collection of distinct, autonomously active nuclei with discrete functions. Ablation and electrical stimulation studies and medical malfunctions have implicated several of these nuclei as central regulatory centers for major autonomic and endocrine homeostatic systems mediating processes such as reproduction, lactation, fluid balance, metabolism, and aspects of behaviors, such as circadian rhythinicity, basic emotions, feeding and drinking, mating activities, and responses to stress, as well as normal development of the immune system (Shepherd, G. M., *Neurobiology,* 3rd ed. Oxford University Press, New York, 1994). Distinct hormones and releasing factors have been associated with some of these nuclei but, at best, the organizations and molecular operations of these structures are only partially understood.

[0005] A substantial portion of a mammal's genetic endowment is dedicated to the function of its central nervous system, as evidenced by the substantial number of mRNAs selectively expressed in the brain (Sutcliffe, J. G., *Ann. Rev. Neurosci.* 11:157-198, 1988). Many of these have been observed to be selectively associated with distinct neural subsets. Existing knowledge of the expression of specific hypothalamic hormones and releasing factors suggests that ensembles of mRNAs selectively associated with discrete hypothalamic nuclei may encode proteins singularly associated with the unique functions of those nuclei.

SUMMARY OF THE INVENTION

[0006] The present invention provides peptides and polypeptides found in the hypothalamus region of the mammalian brain. Preferably, the peptides and polypeptides are enriched in the hypothalamus relative to other regions of the brain. More preferably the peptides and polypeptides are specific to the hypothalamus. One embodiment is the rat polypeptide hypocretin also referred to as, H35 protein or clone 35 protein (SEQ ID NO: 1) and polypeptide analogs thereof having at least one conservative amino acid substitution. Another embodiment is the mouse hypocretin polypeptide (SEQ ID NO: 2) and polypeptide analogs thereof having at least one conservative amino acid substitution.

[0007] The present invention also provides polynucleotides encoding peptides and polypeptides found in the hypothalamus region of the brain. Preferably, the polynucleotides encoding peptides and polypeptides are enriched in the hypothalamus relative to other regions of the brain. More preferably the polynucleotides encoding peptides and polypeptides are specific to the hypothalamus. One embodiment is a polynucleotide chosen from the group consisting of the polynucleotide of SEQ ID NO: 3, a polynucleotide having at least about 95% of its nucleotide sequence identical to the polynucleotide of SEQ ID NO: 3, and polynucleotides hybridizing to the polynucleotide of SEQ ID NO: 3. Another embodiment is a polynucleotide chosen from the group consisting of the polynucleotide of SEQ ID NO: 4, a polynucleotide having at least about 95% of its nucleotide sequence identical to the polynucleotide of SEQ ID NO: 3, and polynucleotides hybridizing to the polynucleotide of SEQ ID NO: 4.

[0008] Also provided are vectors for the expression of the novel polynucleotides operably linked to control sequences capable of directing the production of the novel polypeptides in suitable host cells.

[0009] In other aspects this invention provides pharmaceutical compositions of the polynucleotides, polypeptides and peptides, antibodies to the peptides and polypeptides as well as compositions thereof. This invention also provides assay methods and kits for practicing the methods, and methods for using the polynucleotides, peptides and polypeptides for diagnostic and therapeutic purposes.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] In the Drawings,

[0011] **FIG. 1** shows the results of subtractive screening, enriched for sequences selectively expressed in hypothalamus. Replicate dot blots on which the indicated masses of plasmid DNA for clones of neuron-specific enolase (NSE), cyclophilin, proopiomelanocortin (POMC), vasopressin, the vector pT7T3D, protein kinase Cδ (PKCδ) and growth hormone (GH) were manually spotted and hybridized with cDNA probes made from cRNA transcribed from the target or subtracted libraries, or an equal mixture of the cerebellum and hippocampus driver libraries. Comparison of the signal intensities for the vasopressin dilution series dots at several levels of autoradiographic exposure suggested a 20-to-30 fold increase in the specific activity of vasopressin cDNA.

[0012] **FIG. 2**. shows the results of cDNA library Southern blotting with clones representative of the four distribution classes. The electrophoretic lanes contain the cerebellum first driver library (D1), the hippocampus second driver library (D2), and the hypothalamus target library (T) cleaved with HaeIII and hybridized with the inserts from clone 35 (Panel A), clone 10 (Panel B), clone 86 (Panel C) and clone 19 (Panel D).

[0013] **FIG. 3** distribution of hypothalamic mRNAs. Northern blots with poly(A)$^+$ RNA isolated from extracts of